Therapeutic drug monitoring of immunosuppressant drugs

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Introduction

Individualizing a patient's drug therapy to obtain the optimum balance between therapeutic efficacy and the occurrence of adverse events is the physician's goal. However, achieving this goal is not always straight forward, being complicated by within and between patient variability in both pharmacokinetics and pharmacodynamics. In the early 1960s new analytical techniques became available allowing the measurement of the low drug concentrations seen in biological fluids during drug treatment. This offered the opportunity to reduce the pharmacokinetic component of variability by controlling drug therapy using concentrations in the body rather than by dose alone. This process became known as therapeutic drug monitoring (TDM) [1].

For a drug to be a suitable candidate for therapeutic drug monitoring it must satisfy the following criteria:-

√ There should be a clear relationship between drug concentration and effect.

√ The drug should have a narrow therapeutic index; that is, the difference in the concentrations exerting therapeutic benefit and those causing adverse events should be small.

√ There should be considerable between-subject pharmacokinetic variability and, therefore, a poor relationship between dose and drug concentration/response.

√ The pharmacological response of the drug should be difficult to assess or to distinguish from adverse events.

The immunosuppressive drug cyclosporin satisfies all four of these criteria and, despite over 16 years of clinical use with therapeutic drug monitoring, there is still no firm consensus on the best way to use the drug. In addition, the number of available agents for use as immunosuppressants has more than doubled in recent years and the range of diseases in which these drugs are used has also widened [2]. The purpose of this review is to examine the current strategies in use for the therapeutic drug monitoring of immunosuppressant drugs [3] and to discuss some of the factors that impinge on the monitoring of these drugs.

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Azathioprine, steroids, anti-lymphocyte globulin, and OKT3

The combination of azathioprine and prednisolone was responsible for making clinical transplantation viable [4]. With the addition of anti-lymphocyte globulin [5] (ALG or ATG if human thymocytes instead of human lymphocytes are used to immunise the animal host) they formed the basis of immunosuppression in the early years of transplantation and these drugs are still in widespread use today. Monitoring the blood or plasma concentration of these drugs is not considered worthwhile as they all have relatively wide therapeutic indices. The three agents are generally given in fixed doses and are not subjected to therapeutic drug monitoring.

However, a case can be made for the measurement of the activity of the enzyme thiopurine methyltransferase (TPMT) as an adjunct to azathioprine therapy [6]. Azathioprine is not directly immunosuppressive, since it must be metabolised first to 6-mercapto-purine, then by TPMT to 6-methyl-mercapto-purine and then on to the pharmacologically active 6-thioguanine nucleotides. The expression of the enzyme TPMT is inherited in an autosomal co-dominant fashion and consequently varies widely within the population [7] with 11% of the Caucasian population heterozygous and 0.3% homozygous with respect to TPMT deficiency [8]. Potentially fatal complications could be avoided if TPMT activity was monitored in erythrocytes [9]. The therapeutic drug monitoring of azathioprine in cancer chemotherapy is outside the scope of this article but has been reviewed recently in this journal by Lennard [10].

OKT3 (muromonab-CD3) is a mouse-monoclonal antibody directed against the CD3 complex on T cells [11]. When complexed with its antigen, the antibody prevents the initiation of signal transduction and blocks all T cell function [12]. In a pilot study using OKT3 serum concentrations as a guide to therapy in kidney transplant patients excellent results were reported for the prevention of early graft rejection [13]. Although there is a correlation between OKT3 concentration and T cell killing the relationship is complicated by the patients' antibody response to murine-derived protein [14]. In another study using flow cytometry measurements to monitor OKT3 therapy the authors not only measured OKT3 concentration but also anti-OKT3 antibody

concentration and the number of $CD3^+$ cells (the therapeutic target of OKT3) [15]. Although the authors' conclusions were positive about the use of flow cytometry for monitoring, their over all conclusions were that '*this treatment cannot protect against acute cellular rejection due to the presence of a dimly positive $CD3^+$ population*'. In those centres using muromonab-CD3, TDM for OKT3 is not in widespread use.

Cyclosporin

In the early days of kidney transplantation, Calne gave the 2 year graft survival rate for cadaveric renal transplantation in the USA as less than 18% [16]. Five years later, in 1974, the same author reported that graft survival at 2 years, had risen to over 50% [17]. The change was due to better surgical technique and improved patient management since the main drug therapies, azathioprine and prednisolone, had not changed. Twenty-one years later, in those patients receiving that drug combination, the 2 year graft survival was still only $\approx$60% [18]. However, with the discovery [19, 20] and use of cyclosporin for immunosuppression the 2 year graft survival for the majority of patients is now better than 80% [18]. Long term graft survival has also improved; after the first year from transplant, the estimated graft half-life in patients on cyclosporin alone is 30 years compared with 10.5 years in those patients maintained on azathioprine and steriods [18].

While the introduction of cyclosporin resulted in a marked improvement in graft survival, its use was not without problems. From the initial discovery of cyclosporin [21] to the present day [22], the absorption of the drug has been problematical [23]. Variable absorption and a narrow therapeutic index (the drug causes irreversible kidney damage when given in too high a dose [24]) has resulted in the measurement of cyclosporin blood concentrations so that the dose of the drug can be tailored to the patient to maximise therapeutic efficacy while minimising toxicity [25]. In the past, the utility of cyclosporin therapeutic drug monitoring has been reduced by, choice of sample matrix for monitoring [26, 27], lack of assay specificity [28], inconsistent assay performance [29], the variable absorption of the drug from the original formulation (Sandimmun®) [30] and the poor correlation between trough concentration and clinical effects [31]. Over time, the majority of these problems have been addressed [32]; blood, and not plasma or serum, is the chosen matrix for measurement [33, 34], assays are now more selective for the parent compound [35, 36], most laboratories participate in proficiency testing [37] and the drug has been re-formulated (Neoral®) to improve absorption [38–40]. However, trough whole blood concentration remains an imperfect measure of the total exposure to cyclosporin during a dose interval [3].

In the Sandimmun® era substantial efforts were made by Kahan and co-workers to characterize the relationship between area under the concentration-time curve for cyclosporin (AUC) and clinical events including patient outcome [41]. Although many acknowledge the advantages of AUC monitoring, it has failed to gain widespread acceptance because of the practical difficulties, both for the patient and clinician, in implementing AUC measurements [42]. The advent of the microemulsion formulation of cyclosporin, Neoral®, with its improved pharmacokinetic characteristics [43], provides an opportunity to simplify the measurement of AUC [42]. Previous studies with Sandimmun® have shown that the concentrations of three blood samples, drawn at specific times, can be used to derive an accurate estimate of cyclosporin AUC [44]. For Neoral® a similar degree of accuracy in the prediction can be achieved by two optimally timed blood samples [45]. Prospective studies are now underway to compare pre-dose concentration monitoring with sparse, or limited sampling AUC monitoring.

However, pre-dose, trough concentration and AUC monitoring are not the only options for the therapeutic monitoring of cyclosporin. For some time, Cantarovich and co-workers have been advocating the use of a single timed sample 6 h after dosing [46]. In a prospective study of heart transplant patients comparing pre-dose and 6 h cyclosporin concentration with control cyclosporin therapy, the use of the 6 h value resulted in a 30% lower dose of the drug with the same effectiveness in preventing rejection, and similar cardiac and renal function as seen in those dosed using the pre-dose concentration [47]. These authors have also reported a good relationship between 6 h cyclosporin concentrations and efficacy in non-infectious uveitis [48] and other auto-immune diseases [49].

Another option that is being promoted for monitoring is the use of the cyclosporin blood concentration at 2 h post-dose (*C*2). The rationale for this comes from the observation made during the clinical development of Neoral® that in liver transplant patients there is an inverse relationship between the incidence of rejection and the maximum blood cyclosporin concentration (C_{max}) [50–52]. As yet, the use of *C*2 monitoring has only been tested in a small open-labeled trial but the initial results look promising [53]. The opportunities presented by new therapeutic drug monitoring strategies to optimise cyclosporin therapy have been the subject of a recent consensus meeting [54].

Tacrolimus

Tacrolimus (previously known as FK506), like cyclosporin, has been shown to be an effective immunosuppressive

for the prevention of organ rejection after transplantation and, like cyclosporin, too much drug is associated with toxicity and too little with rejection. Again, like cyclosporin, whole blood concentration measurements are used for the monitoring of tacrolimus therapy [55]. Initial clinical trials did not include concentration monitoring and patients often experienced neuro- and nephrotoxicity [56].

The pharmacokinetics of tacrolimus are highly variable [57]. Since tacrolimus shares many of the pharmacokinetic and pharmacodynamic problems associated with cyclosporin the rationale for therapeutic drug monitoring is similar. An early observational study correlating concentration and effect failed to show a significant difference between the blood concentration in those kidney transplant patients who did not experience rejection and those who did [58]. However other, more statistically rigorous, studies have shown, in kidney and liver transplant patients, significant associations of low tacrolimus concentrations with rejection and of high concentrations with nephrotoxicity [59]. Although the feasibility of a limited sampling scheme to predict AUC has been demonstrated [60], as yet, trough, or pre-dose, whole blood concentration monitoring is still the method of choice. Unlike cyclosporin, there is no move towards the use of other timed samples or AUC monitoring. This may, in part, be due to the high correlation between trough concentration and C_{max} or AUC [61].

Mycophenolate mofetil (MMF)

This is the morpholinethylester of mycophenolic acid (MPA) and acts as a pro-drug for that compound [62]. When given orally to man, MMF undergoes rapid and complete absorption and is hydrolyzed, pre-systemically, to MPA, with no MMF measurable in plasma [63]. The drug reduces both B and T cell proliferation by inhibition of *de novo* guanine nucleotide production [64]. Since, unlike cyclosporin and tacrolimus, both B and T cells are inhibited it has been suggested that MMF may be effective against both acute and chronic rejection [65]. In man, MPA is metabolised in the liver to MPA β-glucuronide (MPAG), an inactive metabolite which is present in plasma at approximately 40-fold higher concentrations than MPA. The MPA glucuronide was thought to be the only metabolite of MPA but, recently, this view has been challenged by comparative results of analyses using high performance liquid chromatography and enzyme immunoassay [66]. These showed a discrepancy between the assays and this was traced to previously unknown, possibly active, metabolites of MPA. The significance of these findings is being assessed [67].

Although the action of the active moiety, MPA, has been known for over 25 years [68], the amount of published data in peer reviewed journals, relating concentration to effect, is limited. Large scale multi-centre, double-blind, randomised, controlled studies in renal transplant patients have shown the effectiveness of MMF in the suppression of acute, biopsy proven, rejection when used in combination with cyclosporin and steroids [69]. Logistic regression has been used to relate the AUC and C_{max} of plasma MPA to the incidence of rejection in renal transplant patients and has demonstrated a highly statistically significant relationship [63]. The results of the logistic regression and data from other trials [70] suggest that low plasma MPA AUC is a significant risk factor in developing rejection [71]. These data have been confirmed by the results of a randomised concentration-controlled study of MMF in renal patients [72]. The link between high MPA concentrations and adverse effects has not been characterised.

The rôle of TDM in MMF therapy has yet to be established. Some authors believe that the inter-individual pharmacokinetic variability is low and therefore the utility of TDM in the majority of patients would be limited [70]. Whereas others, using the same data, believe that the inter-individual pharmacokinetic variability is high and that TDM may have a worthwhile function in the control of MMF therapy [73]. Support for the latter view comes from a study of 30 *de novo* heart transplant patients receiving tacrolimus and MMF in which the dose of MMF was adjusted to maintain the MPA trough plasma concentration between 2.5 and 4 $mg\,l^{-1}$ [74]. These patients were rejection free at 6 months post-transplant and their MMF dose ranged between 0.5 and 6 $g\,day^{-1}$ to achieve trough concentrations within a target range.

Recent consensus guidelines are circumspect about recommending TDM for the control of MMF therapy [75]. The guidelines suggest that TDM should be used to establish that adequate MPA concentrations are achieved soon after surgery and that it could be useful in cases of adverse reaction to MMF. These guidelines were written from the view point of MMF as secondary immunosuppression to cyclosporin or tacrolimus. However, in a recent study it has been shown that MMF can be used successfully as a primary immunosuppressant [76] and if MMF is to be used as mono-therapy the rôle of MPA TDM may assume more importance.

Sirolimus

This is a macrolide antibiotic produced by *Streptomyces hygroscopicus*, a fungus isolated originally from a soil sample from Easter Island (Rapa Nui). Sirolimus (previously known as rapamycin), although similar in structure to tacrolimus, exerts its immunosuppressant effect *via* a different mechanism [77] and at another point in the cell cycle [78]. The initial clinical trials were with the drug

in combination with cyclosporin and were concentration controlled [79]. These trials confirmed the clinical efficacy of the drug [80, 81]. A recent review of the pharmacokinetics of sirolimus suggested a therapeutic range, based on animal allo-transplant data, of 5 to 10 $\mu g\,l^{-1}$ in whole blood [82]. However, the only consensus guidelines published on the therapeutic monitoring of sirolimus concluded that there was not enough information available about the clinical use of the drug to make recommendations [83].

Daclizumab and basiliximab

The binding of interleukin 2 (IL-2) to its receptor on antigen activated T cells stimulates clonal proliferation of the T cells that mediate organ allograft rejection [84]. The receptor complex is made up of at least three sub-units (α, β and γ) and, of these, only the α sub-unit is thought to be specific to IL-2 [85]. This presents a potential therapeutic target for specific immunosuppressive therapy and it has been exploited by a series of monoclonal antibodies raised against the IL-2 receptor's α sub-unit (IL-2Rα). Two such antibodies are currently available. One is a molecularly engineered human IgG1 incorporating the antigen-binding regions of a murine monoclonal antibody (daclizumab [86]) against IL-2Rα. The other is a murine-human chimeric antibody (basiliximab [87]) to IL-2Rα. Both have been used successfully for the prevention of acute rejection following renal transplantation [88, 89]. The therapeutic index of these antibodies is wide, neither antibody being associated with major adverse effects and, since the half life of both antibodies is long, daclizumab ($t_{1/2} \approx 6$ days [90]) is given as five, and basiliximab ($t_{1/2} > 20$ days [91]) as two, weight related intra-venous doses in the first 8 weeks following transplantation. Thus, there appears little to be gained from therapeutic drug monitoring for these agents [92, 93].

The 'therapeutic' range

In a survey of the therapeutic ranges used by 21 transplant centres to guide cyclosporin maintenance therapy in kidney transplant patients there was a six fold range in the concentrations considered effective and a three fold range in those considered toxic [94]. There was also considerable variation in the width of the therapeutic 'window'. Although some of this variation must come from the different combinations of immunosuppressant drugs used and differences in assay methodology [35] and, perhaps, specificity [95], much of the variation is due to the empirical way that these ranges have been derived.

Early attempts to set target ranges to achieve efficacy, while avoiding toxicity, were based on simple clinical observations in patients who had undergone kidney transplantation [31]. More systematic approaches have been made by the retrospective statistical analysis of large volumes of patient data gathered over a period of years [96]. However, the ranges arrived at are biased by subjective judgments; a more objective approach has been taken by Perna *et al.* [97]. These authors used logistic regression to determine the most probable concentrations for the occurrence of rejection and toxicity in renal transplant patients. This approach has also been used by Nicholls [71] to determine the therapeutic range for mycophenolic acid and is applicable to the other immunosuppressant agents and their combinations.

Morris [98] has called the whole concept of the therapeutic range into question. This author suggests that the concept should be abandoned as it puts too much emphasis on achieving the desired numbers rather than treating the patient. He favours a single concentration approach in which clinicians aim, initially, to dose a patient to achieve a set target concentration. The target is then individualised for that patient based on the number of rejection episodes, occurrence of toxicity, concomitant medication, etc. This has the advantage that pharmacokinetic variability is controlled by the target concentration and pharmacodynamic variability is dealt with by tailoring the target to the patient.

Some of the problems alluded to by Morris could be addressed if the boundaries of the therapeutic ranges were not seen as yes-no cut-off points. To do this the performance characteristics of the concentrations need to be seen in terms of a diagnostic test for determining the probability of drug-effectiveness or toxicity [99]. This, again, requires better information to be available to the clinician so that the blood or plasma concentrations can be interpreted within a Bayesian statistical framework and informed decisions can be made [100].

Assay methodology

Cyclosporin The correct measurement of cyclosporin has been the subject of many publications [26, 27, 101] and reviews [37, 95]. Currently four commercial companies are producing eight different immunoassay assay systems for the measurement of cyclosporin in whole blood (Table 1). In addition, a number of laboratories are using high performance liquid chromatography (h.p.l.c.) to measure the drug. Using h.p.l.c., it is possible to separate the parent compound from metabolites and for this reason this technique has long been considered the 'gold standard' in cyclosporin measurement, particularly when coupled with mass-spectrometry. However, although h.p.l.c. is specific for cyclosporin, the technique can

Table 1 Available assays for the measurement of cyclosporin and abbreviations and manufacturers.

Abbreviation	*Assay*
H.p.l.c.	High performance liquid chromatography.
TDxsp	Fluorescence polarisation immunoassay with monoclonal specific antibody for the Abbott TDx® analyser.
TDxns	Fluorescence polarisation immunoassay drug and metabolite with polyclonal non-specific antibody for the Abbott TDx® analyser.
AxSYM	Fluorescence polarisation immunoassay with monoclonal specific antibody for the Abbott AxSYM® analyser.
r.i.a.sp	Radioimmunoassay with monoclonal specific antibody, DiaSorin Cyclo-Trac-SP.
r.i.a.ns	Radioimmunoassay with monoclonal non-specific antibody, DiaSorin Cyclo-Trac-NS.
EMIT	Homogeneous enzyme immunoassay, methanol extraction, EMIT Dade Behring.
EMITgl	As EMIT but with patented 'green liquid' extraction system, EMIT Dade Behring.
CEDIA	Homogeneous enzyme immunoassay, CEDIA Roche Diagnostics (no results shown, See Schütz *et al.*[106]).

suffer from poor precision and can give spurious results due to interference from other sources [102].

Of the eight immunoassay variants, two are non-specific and cross-react, markedly, with the metabolites of cyclosporin, (Figure 1). The Abbott TDx® Drug and Metabolite assay uses a polyclonal antibody and produces results that are approximately 3 to 5 times those of h.p.l.c. whereas the DiaSorin CYCLO-Trac-NS radio-immunoassay uses a monoclonal non-specific antibody and gives results about 5 to 7 times higher than h.p.l.c. The ratio of the non-specific assays to h.p.l.c. changes with the metabolite : parent compound ratio in the blood and, therefore, will vary with transplant type and time after transplant. The results of the non-specific assays have a poor correlation with clinical events [103].

The other six immunoassays are regarded as 'specific' for the parent drug but do, to a limited extent, cross-react with some of the metabolites of the drug and, therefore, do not necessarily give the same result for a given sample (Figure 2). Although the differences between the results of the 'specific' assays are due, in part, to the different cross-reactivities of the anti-bodies used, some of the differences result from incorrect calibration [104]. This can be seen clearly with the measurement of spiked samples (Figure 3). (It is interesting to note that for one of the manufacturers the results of their three different assays do not agree). These differences in measurement accuracy do not seem to affect the clinical usefulness of the assays [103] but do add to variability of reported concentrations in the literature [105] and have an impact on the local target ranges [94]. However, in clinical conditions where the cyclosporin metabolite load in blood is high, for example, in liver transplant patients immediately post-transplant, h.p.l.c. is the only method that can accurately measure the parent compound [106].

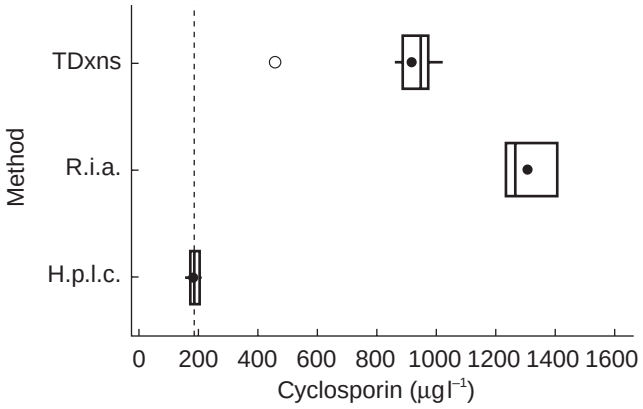

Figure 1 Measurement of cyclosporin in an aliquot of pooled blood samples from heart transplant patients receiving the drug. The results are shown as Box and Whisker. The line is drawn across the box at the median. The left of the box is at the first quartile (Q1), and the right is at the third quartile (Q3) value. The whiskers are the lines that extend from the left and right of the box to the adjacent values within $\pm 1.5 \times (Q3 - Q1)$, values outside the whiskers are plotted as circles. The mean result is shown as a solid circle. plots for h.p.l.c. (11 centres), TDx non-specific (19 Centres) and r.i.a. non-specific. The dotted line is at the median value, 190 $\mu g\,l^{-1}$, for h.p.l.c. (data from the Cyclosporin International Proficiency Testing Scheme [114]).

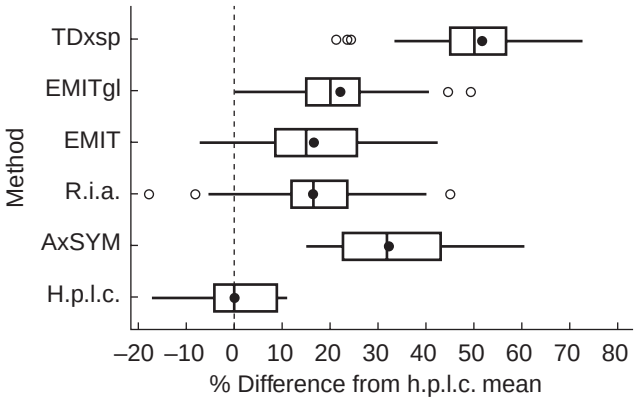

Figure 2 Measurement of cyclosporin in an aliquot of pooled blood samples from heart transplant patients receiving the drug. The results are shown as Box and Whisker. The line is drawn across the box at the median. The left of the box is at the first quartile (Q1), and the right is at the third quartile (Q3) value. The whiskers are the lines that extend from the left and right of the box to the adjacent values within $\pm 1.5 \times (Q3 - Q1)$, values outside the whiskers are plotted as circles. The mean result is shown as a solid circle. plots for percentage difference from the median result for h.p.l.c. (11 centres), AxSYM, r.i.a.sp, EMIT, EMITgl and TDxsp.

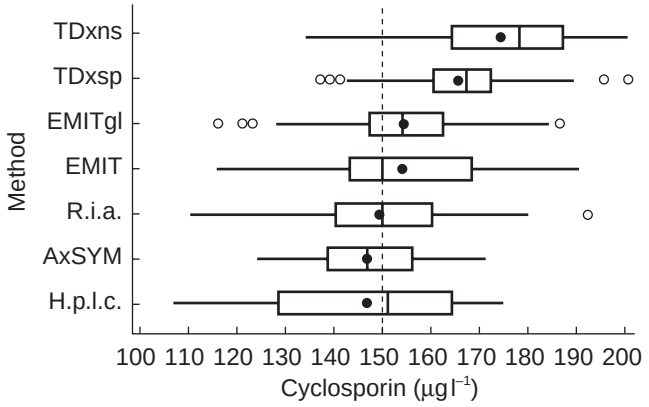

Figure 3 Measurement of cyclosporin in an aliquot of drug free blood to which 150 $\mu g\,l^{-1}$ of cyclosporin had been added. The results are shown as Box and Whisker. The line is drawn across the box at the median. The left of the box is at the first quartile (Q1), and the right is at the third quartile (Q3) value. The whiskers are the lines that extend from the left and right of the box to the adjacent values within $\pm 1.5 \times (Q3 - Q1)$, values outside the whiskers are plotted as circles. The mean result is shown as a solid circle. plots for h.p.l.c. (11 centres), AxSYM, r.i.a.sp, EMIT, EMITgl, TDxsp and TDxns.

Tacrolimus The concentrations of tacrolimus seen in the blood of stable renal transplant patients is low, $<30\ \mu g\,l^{-1}$, and this makes the measurement of the drug difficult. In-house ELISA, commercial ELISA (DiaSorin), microparticulate enzyme immunoassays, and h.p.l.c.-MS [107] methods have been available. The majority of laboratories monitoring tacrolimus use the commercial microparticulate enzyme immunoassay (MEIA, Abbott Laboratories) which measures the drug within the range 3 to 30 $\mu g\,l^{-1}$ and cross-reacts to a small degree with the metabolites of tacrolimus [108].

Sirolimus Therapeutic drug monitoring of sirolimus is still under investigation. The assays that are in current use are based on h.p.l.c. using either ultra-violet or mass spectroscopy to detect the drug. However, a commercial MEIA (Abbott Laboratories) is being tested for widespread, routine, measurement of the drug [109].

Receptor assays Cyclosporin, tacrolimus and sirolimus bind to a group of widely occurring proteins, the immunophilins. The two major immunophilins are cyclophilin, which binds cyclosporin, and FK-binding protein 12, which binds tacrolimus and sirolimus. Receptor assays have theoretical advantages over current immunoassays in that binding is associated with pharmacological activity so that only active drug or active metabolite concentration is measured [110]. However, this contention still remains to be proved and, as yet, these assays are not in routine clinical use.

Mycophenolic acid Compared with the other immunosuppressant drugs in current use, the plasma concentration of MPA is much higher and this makes h.p.l.c. measurement of the drug straightforward. However, the major glucuronide metabolite of MPA complicates the assay as it elutes much later than the parent drug and this leads to extended run times. A commercial homogeneous enzyme immunoassay (Dade Behring) is available which is capable of accurate and precise measurement of the drug in the concentration range 0.5 to 15 $mg\,l^{-1}$. This assay does not cross react with the major glucuronide of MPA but does give results marginally higher than h.p.l.c. because of cross reactivity to minor MPA metabolites [66].

Proficiency testing

The difficulties in measuring blood cyclosporin concentration led to the development of a proficiency-testing scheme for laboratories providing monitoring services for the drug [29]. The Scheme has been in existence for over 14 years and has over 400 participating centres world-wide. The Scheme circulates three blood samples *per* month to each participating centre and the measurements made on these samples enable laboratories to benchmark their analytical performance against currently available best practice [111]. The manufacturer of cyclosporin, Sandoz AG (now Novartis AG), initially funded the Scheme to promote measurement accuracy of the drug. However, the individual centres, the drug manufacturer and TDM reagent suppliers, now fund it.

In a similar fashion Fujisawa GmbH, the makers of tacrolimus [112], have funded a scheme for that drug and Dade Behring, manufacturer of a kit for the measurement of MPA, have funded a mycophenolate proficiency scheme [113]. These schemes have 200 and 38 members, respectively. A sirolimus proficiency testing scheme was introduced in January 1999 [114]. The rôle and rationale for proficiency testing schemes in immunosuppressive drug monitoring has recently been reviewed [115].

Pharmacodynamic monitoring

Blood or plasma drug concentration measurements are only a surrogate for effect. Their use would be unnecessary were it possible to measure the immunosuppressive action of these drugs directly. However, defining the pharmacodynamic target for monitoring is not an easy task. For example, in a recent study investigating immunological monitoring of azathioprine the authors examined multiple subsets of peripheral blood lymphocytes, natural killer activity, the serum concentrations of IgG, IgM, interferon β (IFN β), tumour necrosis factor α (TNF α), interleukin 2 (IL-2), soluble interleukin 2 receptors (sIL-2R), interleukin 6 (IL-6) and the soluble adhesion molecule

sICAM-1 [116]. There is a danger that one surrogate will be replaced by another.

Cyclosporin and tacrolimus inhibit T-cell proliferation, which is thought to result from their inhibition of calcineurin [117]. This is a serine-threonine phosphatase that plays an essential rôle in intra-cellular, calcium-dependent, signal transduction [118]. Inhibition of calcineurin in activated T cells reduces the translocation of the cytoplasmic sub-unit of the nuclear factor to the nuclear sub-unit and, hence, impairs the transcription of genes for many of the cytokines essential for the rejection response [119]. For this reason calcineurin has been the primary focus of pharmacodynamic monitoring for cyclosporin. In a study of 62 renal transplant patients the measured calcineurin activity in leukocytes was half that of controls [120]. The trough cyclosporin concentration was inversely related to the calcineurin activity. Since tacrolimus acts on the same target, calcineurin would also be applicable for monitoring that drug. However, the measurement of calcineurin activity is technically challenging and a much simpler procedure would be required before it could be used in the routine setting. In any case, further studies are needed to confirm that calcineurin activity correlates better with patient response than simple blood concentrations.

Mycophenolic acid exerts its immunosuppressive action by inhibition of inosine monophosphate dehydrogenase (IMPDH) and, thereby, blocking *de novo* purine biosynthesis in lymphocytes [121]. An assay has been developed for the measurement of IMPDH activity in whole blood to measure drug effect rather than concentration [122]. As yet there are few published data to support the use of this assay, but initial studies suggest a correlation between IMPDH activity and clinical events [123].

Sirolimus is thought to exert its immunosuppressive activity by blocking the phosphorylation and activation of the P70 S6 kinase that is involved in cell signalling, and this prevents, or reduces, lymphocyte proliferation [124]. The measurement of P70 S6 activity is, therefore, a prime target for pharmacodynamic measurements. An assay has been developed for its measurement in whole blood and studies are ongoing to determine its utility in clinical practice [125].

Antithymocyte globulin (ATG) has been monitored in renal transplant patients using the effect of ATG on subsets of T lymphocytes [126]. In this study, the author identified the $CD3^+$ lymphocytes as a pharmacodynamic marker of ATG response. The dose of ATG administered to patients was titrated to maintain the patients' absolute $CD3^+$ lymphocyte count at ≈ 50 cells μl^{-1} of blood. Forty-four patients who were treated in this way for steroid resistant rejection had significantly less serious viral infections than 10 patients who were treated on a fixed dose ATG regimen, but their 1 year graft survival was not compromised. Although not a formal pharmacoeconomic study, the author also noted that there was a net saving of £1600 ($2500) *per* patient.

Benefits of monitoring

Evidence-based medicine is sadly lacking in the area of drug monitoring [127]. Although the perception of therapeutic drug monitoring is that it is beneficial, and aids patient management, there is little hard evidence to support that view. Outside the field of immunosuppressive drugs there are numerous articles, detailing predominantly retrospective studies, which suggest that TDM has a useful and cost effective rôle in monitoring therapy [128, 129]. However, as the authors of a recent review of clinical pharmacokinetics point out, there is little evidence to support the effect of TDM on true patient outcomes [128].

For the immunosuppressive drugs some positive evidence for TDM is given by the results of prospective concentration-controlled clinical trials [130]. A concentration-controlled trial is one in which patients are dosed to achieve pre-assigned target concentrations and, therefore, the results can be assessed in terms of drug concentration and response rather than dose and response. This type of trial has several benefits over the randomized dose-controlled trials [131] and can provide the basis for future TDM decisions.

In a study of tacrolimus, patients were randomly allocated to be targeted at low, medium or high drug blood concentrations [132]. Although there was no difference in the incidence of rejection or toxicity in the three groups in the first 42 days post transplant, logistic regression demonstrated a clear relationship between concentration and effect (Table 2). In a study of similar design using mycophenolate mofetil, patients were randomly allocated to a high, medium or low mycophenolic acid AUC [72]. Again there was a clear concentration related effect of the drug with rejection in the low, medium and high groups of 26, 9 and 6%, respectively.

Conclusion

At present the majority of drug regimes in use for transplantation are based on cyclosporin and excellent

Table 2 Incidence of toxicity and rejection by whole blood tacrolimus concentration [132].

	Tacrolimus ($\mu g\ l^{-1}$)		
	<5	5–15	>15
Rejection (%)	34	17	5
Toxicity (%)	0	34	54

results can be obtained in solid organ transplantation using this drug. This is also true in the treatment of autoimmune diseases [2]. However, cyclosporin's dominance is being challenged as the number of immunosuppressive agents increases and clinical experience is gained with other drugs. We are entering an era in which combination therapy will be the norm and clinicians will tailor the immunosuppression to the characteristics of the individual patient changing dose and drugs as time progresses and conditions change [133]. In addition, generic [134] and other new formulations of cyclosporin [135] will be available in the near future and these together with the new drugs under clinical development, such as FTY720 [136] and SDZ-RAD [137], and the possibilities of xenotransplantation [138] present future challenges which will add to the complexity of TDM.

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